THE MEIOSTAGMIN AND EPIPHANIN REACTIONS IN THE DIAGNOSIS OF CARCINOMA.*

W. H. BURMEISTER.

(From the Memorial Institute for Infectious Diseases, Chicago.)

In 1910 Ascoli and Izar[1] first employed the stalagmometer in the diagnosis of malignant tumors, and in 1912 Rosenthal[2] applied the epiphanin reaction to the differentiation of carcinoma proteins from normal organ proteins and suggested its application in the diagnosis of carcinoma.

Previous to the appearance of Rosenthal's work, I had made attempts to adapt the epiphanin reaction to the diagnosis of carcinoma. In none of the preliminary experiments, however, were results obtained which were beyond the range of error as established by the controls. Subsequent to Rosenthal's report, a comparative study of the meiostagmin and epiphanin reactions in a limited series of cases was undertaken. This series consists of 20 cases, in all of which the clinical diagnosis was established without any doubt.

THE MEIOSTAGMIN REACTION.

To obtain a reliable antigen, proved rather difficult. Micheli and Catoretti,[3] and Verson[4] used antigens from pancreas and thyroid tissue. I prepared antigens from human carcinoma, the thyroid gland (both exophthalmic and adenomatous), placenta (washed free from blood), and dog pancreas. Preliminary titrations demonstrated the pancreas antigens to be far more active than those obtained from thyroid, placenta, or even carcinoma tissue, and in the subsequent tests this was the antigen employed. The antigen was prepared by desiccating the tissue, which had been previously rubbed up in a mortar with quartz sand, in a current of air at about 37° C. The powdered desiccate was then treated with methyl alcohol, in the proportion of 1–4, at 50° C. for 24

* Received for publication February 14, 1913.

[1] *München. med. Wchnschr.*, 1910, 57, p. 403.

[2] *Ztschr. f. Immunitätsf.*, 1912, 15, p. 37.

[3] *München. med. Wchnschr.*, 1910, 57, p. 1122.

[4] *Wien. klin. Wchnschr.*, 1910, 23, p. 1102.

hours with frequent shakings. This was then filtered while still hot through a Schleicher and Schuell filter No. 590. After cooling, the filtrate was filtered again. This extract was then titrated in water emulsions of various dilutions with normal sera until a dilution was obtained that would not cause an increase of more than one drop as determined by a Traube's stalagmometer. All sera were 24 hours old. The technic as revised by Ascoli and Izar[1] was followed. Two control sera, one noncarcinomatous and one carcinomatous, were tested with each serum. The reactions were completed by heating the serum and antigen mixtures (9–1) in a water bath at 50° C. for one hour. The graduations on the stalagmometer were such that one drop of the serum dilutions in general averaged 13 divisions of the scale. Ten of the 11 cases of malignant tumors in this series were carcinomas; one was a multiple myeloma. In all of these, the surface tension of the serum was decreased by the action of the antigen. In all but two, the decrease caused an increase of more than one drop. In only 5 of the sera was the increase greater than 2 drops.

Ascoli originally pronounced only such reactions positive as presented an increase of two drops or more. Later investigators have considered an increase of less than two drops as positive. Koehler and Luger,[2] working with an acetone lecithin extract as antigen, include an increase as low as 12/13 drop as positive. The tendency to assume as positive an increase of less than two drops or even than one drop, undoubtedly accounts for the varying degrees of success attained by different workers with this reaction. According to Ascoli's standard, only 45 per cent of the tumor cases of my series presented a positive reaction, while 11 per cent of the surely noncarcinomatous cases reacted positively. Were these figures to be revised according to the standard of Koehler and Luger, however, 100 per cent of the tumor cases and 33 per cent of the other cases give a positive reaction. During the course of these experiments, it was accidentally discovered that a very slight trace of soap was capable of decreasing surface tension as determined by the stalagmometer. This also has been noted by

[1] *München. med. Wchnschr.*, 1910, 57, p. 1170, 2129.

[2] *Wien. klin. Wchnschr.*, 1912, 25, p. 1114.

Kelling.[1] Not infrequently the water-antiserum controls presented a slight decrease in surface tension which I was unable to explain. In this series it is interesting to note that the reactions in two cases of myelogenous leukemia were negative, while that of a case of multiple myeloma (myelocytoma) was positive.

THE EPIPHANIN REACTION.

According to Weichardt,[2] the epiphanin reaction depends in part on an acceleration of the rate of diffusion in a solution, when antigen and its specific antibody are introduced. For demonstration a barium-hydrate-sulphuric-acid system is employed. In this system the sulphuric acid is of such concentration as to exactly neutralize an equal volume of saturated barium hydrate solution. Phenolphthaline in combination with a catalytic agent, strontium chlorid, is used as the indicator. Colloidal substances are said to alter the surface tension of the suspension of finely divided barium sulfate particles so as to increase the absorption of H ions. In this way the point of neutralization is shifted accordingly as the amount of absorption is large or small. A definite quantity of the barium hydrate and sulphuric acid is added to definite amounts of antigen and antibody which have previously been allowed to act on each other; the control is obtained by adding the same quantity of barium hydrate and sulphuric acid to the same amounts of antigen and antibody, but before there has been time for a reaction between antigen and antibody to take place. The degree to which the point of neutralization has been shifted in both instances is then determined by titrating with N/1000 H_2SO_4 and the difference between the two determined.

In this manner the variations for three different serum dilutions (10^4, 10^6, 10^8), have been determined and plotted in the form of a curve. Figures on the axis of ordinates represent cubic centimeters of N/1000 H_2SO_4; figures on the abscissae represent the different serum dilutions (10^2=1–100, 10^4=1–10000, etc.). An attempt was made to use the same antigens in the epiphanin reactions as were employed in the meiostagmin reaction. This was done in 5 of the 20 sera which were tested. In the remaining 15

[1] *Arch. f. Verdauungskr.*, 1912, 18, p. 164. [2] *Ztschr. f. Immunitätsf.*, 1910, 4, p. 651.

sera, extracts made with 20 per cent glycerin water from carcinoma of the breast and uterine cervix according to the technic of Rosenthal[1] were employed. The spiral pipette and the four-glass method recommended by Rosenthal[2] were also employed.

Titrations by the four-glass method are made in a series of four beakers of equal dimensions. In beaker 1, are placed 0.1 c.c. of the antiserum and 1 c.c. of the antigen dilution. In beaker 2, are placed 1 c.c. of the antigen and 0.1 c.c. of distilled water. In beaker 3, are placed 1 c.c. of water and 0.1 c.c. of the antiserum

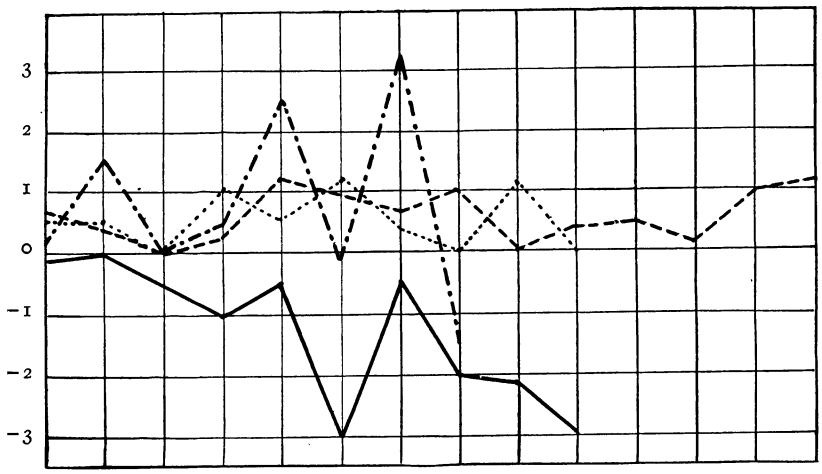


CHART 1.—Epiphanin reaction. Titrations with reagents only.

. . . . . Curve for blind titrations by single-glass method.
– – – – – – " " " " " " " " "
——— " " " " " four " "
– – – – – – " " " " " " " "

Figures on the axis of ordinates represent c.c. of N/1000 H_2SO_4.

dilution. In beaker 4, are placed 1.1 c.c. of distilled water. The contents of the beakers are mixed by gently shaking and then allowed to stand 10 or 15 minutes. Following this, the barium-hydrate-sulphuric-acid system and the indicator are added in equal quantities to each beaker. The contents of beaker 4 are then added to beaker 1 and those of beaker 3 to beaker 2. Beaker 1 and beaker 2 then contain equal quantities of reagents, indicator, water, antigen and antiserum, the only difference being that the

[1] *Ztschr. f. Immunitätsf.*, 1912, 15, p. 37. [2] *Ibid.*, 1912, 13, p. 383.

antigen and antiserum of beaker 1 have had an opportunity to act one on the other before the addition of the chemical system.

A series of blind titrations in which distilled water was substituted for both antigen and antiserum was first made. This series consisted of several hundred titrations to determine the range of error in this reaction. Charts 1 and 2 represent some of the

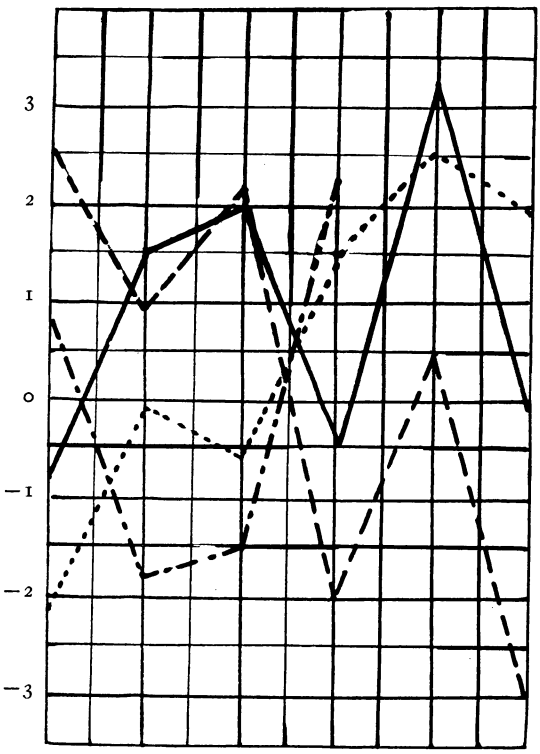


CHART 2.—Epiphanin reaction. Titrations with reagents only. Four curves obtained by four-glass method.

extremes of the range of error in these titrations. During the course of the work von Angerer and Stoetter[1] published a single-glass method by which they are able to decrease their range of error very materially. That this should be possible is readily understood. The four-glass method requires just four times the amount of reagents, serum, and antigen, and four times as much

[1] *München. med. Wchnschr.*, 1912, 59, p. 2035.

manipulation as the single-glass method. The single-glass method employs but one beaker. Its contents and the order of their addition are the same as those of beaker 1 of the four-glass method. It is assumed by von Angerer and Stoetter that the neutral point

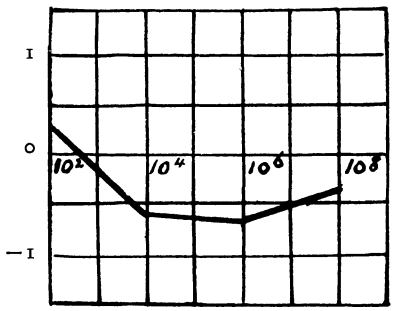


CHART 3.—Epiphanin reaction. Normal serum.

Figures on the abscissae represent different dilutions of serum, 10^2=1–100, 10^4=1–1000, etc. Reactions obtained with Antigen I, a glycerin-water extract of a mammary carcinoma. Four-glass method.

Meiostagmin reactions with same serum and Antigen II, an alcoholic extract of pancreas.

Before heating	49^{+1} drops
After "	49^{+8} "
H_2O control	49^{+2} "

(49^{+1} gtt. = 49 drops and approximately 1/13 of a drop.
49^{+8}gtt. = 49 drops and approximately 8/13 of a drop, etc.)

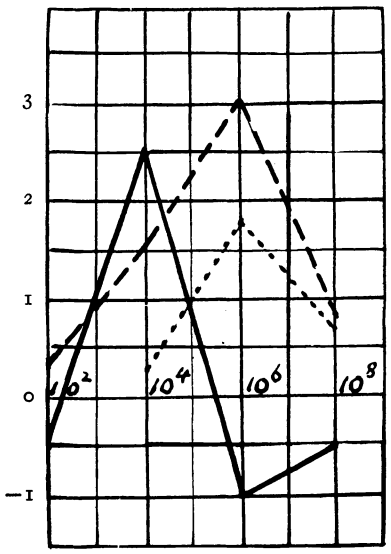


CHART 4.—Epiphanin reaction. Serum from carcinoma of neck.

——— Antigen III (Alcoholic extract of placenta).
. . . . . Antigen IV (Alcoholic extract of thyroid).
- - - - - - - - Antigen II.
Four-glass method.
Meiostagmin reaction with same sera.

Antigen	Before heating	After heating	Control
III	50^{-8} drops	50^{-1} drops	50^{-2} drops
IV	50^{-8} "	50^{-4} "	50^{-2} "
II	50^{-8} "	53 "	50^{-2} "

of the combined contents of beakers 2 and 3 is the same as that for the barium-hydrate-sulphuric-acid system; they assume the latter to be a constant, i.e., 0.

In a series of several hundred blind titrations with the single-glass method, my range of error was but little if any greater than

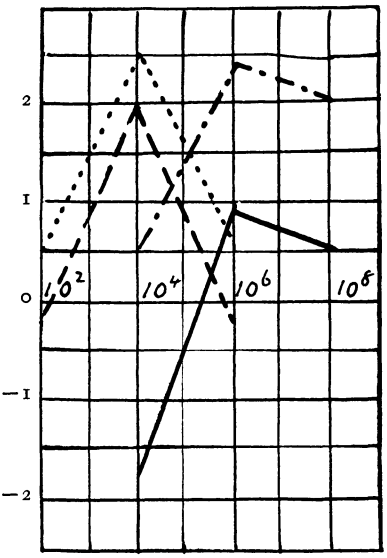


CHART 5.—Epiphanin reaction. Four cases of carcinoma.

———— Mammary carcinoma, two years after operation, recurrence in scar, bone metastases, no cachexia; antigen II; four-glass method.

Antigen	Before heating	After heating	Control
II	50^{-6} drops	54^{-3} drops	50^{-5} drops
III	50^{-6} "	50^{-1} "	50^{-5} "
IV	50^{-6} "	50^{+1} "	50^{-5} "

- - - - - - - - Mammary carcinoma, first noticed 7 months before, age 35 years; no cachexia; antigen II; four-glass method.

Meiostagmin reaction for same serum: antigen II, before heating, 49^{-1} drops, after heating 50^{-2} drops, control, 49^{-1} drops.

. . . . . Mammary carcinoma, 3 years after operation, carcinoma of axillary glands at this time, no cachexia; antigen II; four-glass method.

Meiostagmin reaction for same serum: antigen II, before heating, 49^{-2} drops, after heating 51^{-4} drops, control, 49 drops.

-·-·-·-·-·- Mammary carcinoma, entire right breast and pectoral muscles involved, 67 years, marked cachexia; antigen V, glycerin water extract; of cervix carcinoma; four-glass method.

Meiostagmin reaction for same serum:

Antigen	Before heating	After heating	Control
II	51^{-9} drops	52^{-4} drops	51^{-7} drops
VI	51^{-9} "	51 "	51^{-7} "

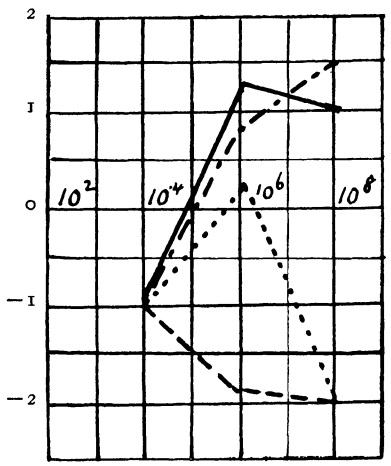


CHART 6.—Epiphanin reactions.

———— Carcinoma of oesophagus, first symptoms of stenosis 1 year ago, age 65, loss of weight 152 lbs., former weight 240 lbs.; antigen I; four-glass method.

Meiostagmin reaction for same serum: antigen II, before heating 50^{-6} drops, after heating 52^{-5} drops, control, 50^{-1} drops.

- - - - - - - - Case of chronic sciatica, age 20 years, well nourished; antigen I; four-glass method.

Meiostagmin reaction for same serum: antigen II, before heating 51 drops, after heating 52^{-6} drops, H_2O control, 51^{-4} drops.

. . . . . Herniotomy, uncomplicated, 5 days after operation, ether anesthesia, well nourished; antigen V; four-glass method.

Meiostagmin reaction for same serum: antigen II, before heating 52^{-9} drops, after heating 52^{-4} drops, control, 52^{-5} drops.

-·-·-·-·-·- Infected finger, axillary adenitis, age 18 years, well nourished; antigen I; four-glass method.

Meiostagmin reaction for same serum: antigen II, before heating 49^{-2} drops, after heating 50^{-5} drops, control, 50^{-8} drops.

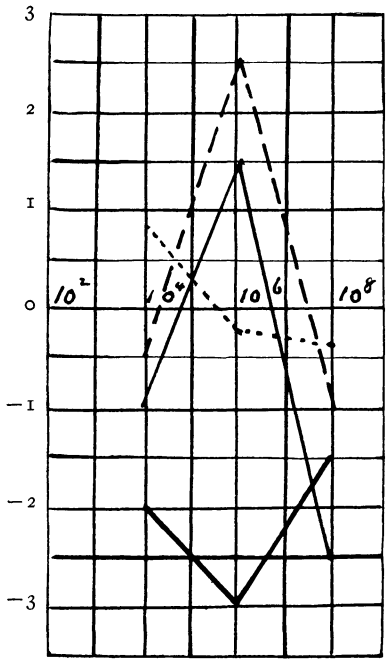


CHART 7.—Epiphanin reactions. Carcinoma of oesophagus, first symptoms of stenosis 4 months ago, loss of weight 104 lbs. in 4 months, present weight 180 lbs.

——— Simultaneous titrations; antigen I; four-glass method.

Meiostagmin reaction, same serum: antigen II, before heating, 48^{-2} drops, after heating, 53^{-2} drops, control, 48^{-4} drops.

- - - - - - - - Same serum, antigen I plus antikenotoxin, four-glass method.

. . . . . Same serum, antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating 48^{-2} drops, after heating, 53^{-2} drops, control 48^{-4} drops.

——— Simultaneous titrations, antigen I, four-glass method.

- - - - - - - - Antigen I plus antikenotoxin, four-glass method.

. . . . . Antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating, 48^{-4} drops, after heating 49^{-3} drops, control, 48^{-1} drops.

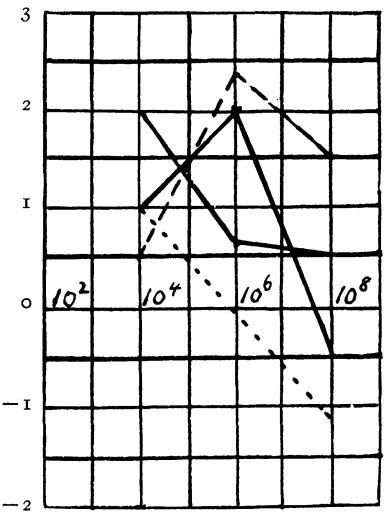
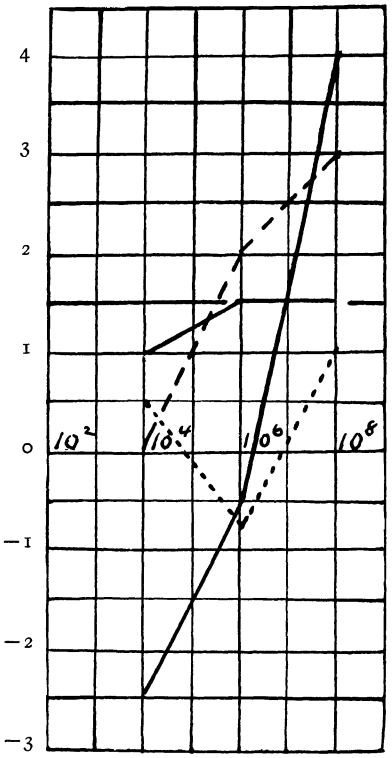


CHART 8.—Epiphanin reactions. Carcinoma of stomach, marked cachexia.



———— Simultaneous titrations, antigen I, four-glass method.

- - - - - - - - Antigen I plus antikenotoxin, four-glass method.

. . . . . Antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating, 48^{-4} drops, after heating, 49^{-3} drops, control, 48^{-2} drops.

CHART 9.—Epiphanin reactions. Carcinoma of stomach, marked cachexia.

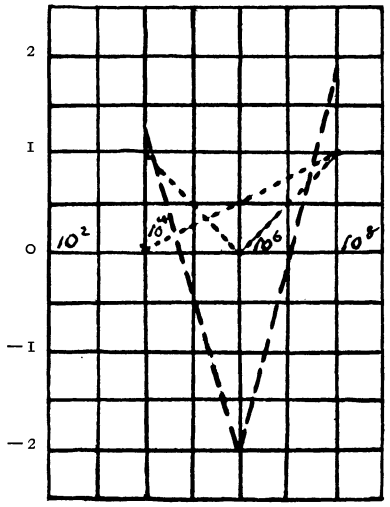


CHART 10.—Epiphanin reactions. Carcinoma of stomach, slight cachexia.

. . . . . Two curves, simultaneous titrations, antigen I, single-glass method.

- - - - - - - - Antigen I plus antikenotoxin, four-glass method.

Meiostagmin reaction of same serum: antigen II, before heating 48^{-4} drops, after heating, 49^{-6} drops, control, 48^{-12} drops.

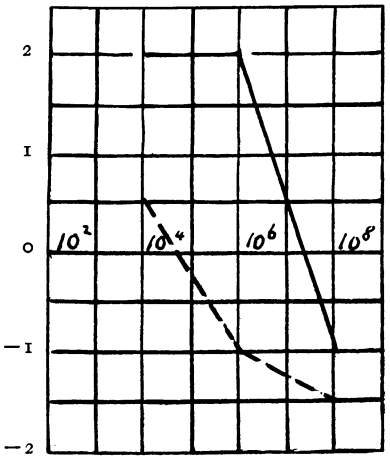


CHART 11.—Epiphanin reactions. Pulmonary tuberculosis, exudative pleuritis, slight cachexia.

——— Antigen I, four-glass method.

- - - - - - - - Same serum, antigen I plus antikenotoxin, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating, 49^{-1} drops, after heating, 50^{-6} drops, control, 49^{-3} drops.

——— Simultaneous titrations, antigen I, four-glass method.

- - - - - - - - Antigen I plus antikenotoxin, four-glass method.

. . . . . Antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating, 48^{-8} drops, after heating, 52^{-6} drops, control, 48^{-8} drops.

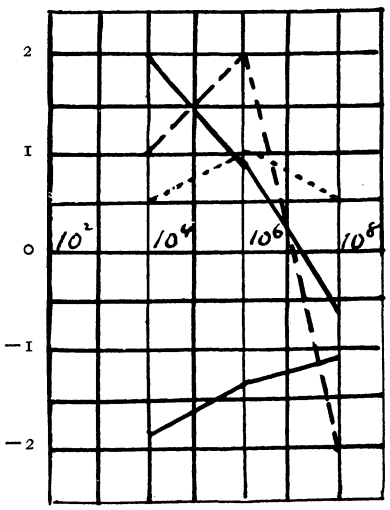


CHART 12.—Epiphanin reactions. Typhoid, 5th week, relapse.

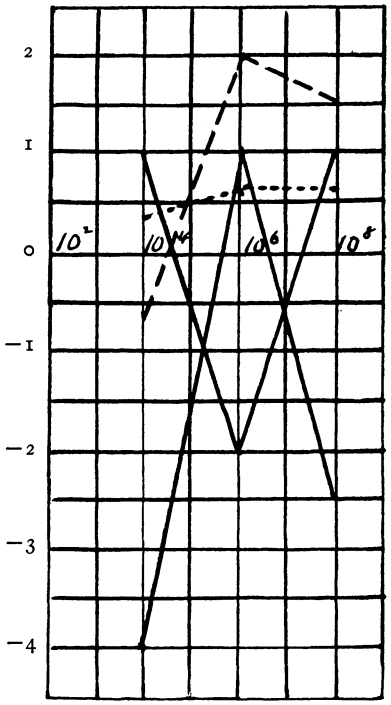


CHART 13.—Epiphanin reactions. Chronic nephritis, uremia.

——— Simultaneous titrations, antigen I, four-glass method.

- - - - - - - - Antigen I plus antikenotoxin, four-glass method.

. . . . . Antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating, 48 drops, after heating, 49^{-4} drops, control, 48^{-2} drops.

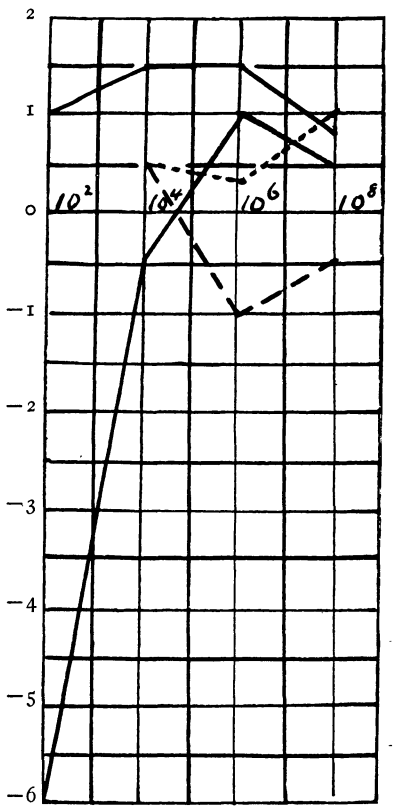


CHART 14.—Epiphanin reactions. Multiple myeloma of clavicle, ribs, vertebrae, pelvic bones, long bones, and skull, extreme cachexia.

——— Simultaneous titrations, antigen I, four-glass method.

- - - - - - - - Antigen I plus antikenotoxin, four-glass method.

. . . . . Antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating, 48^{-3} drops, after heating, 50^{-2} drops, control, 49^{-9} drops.

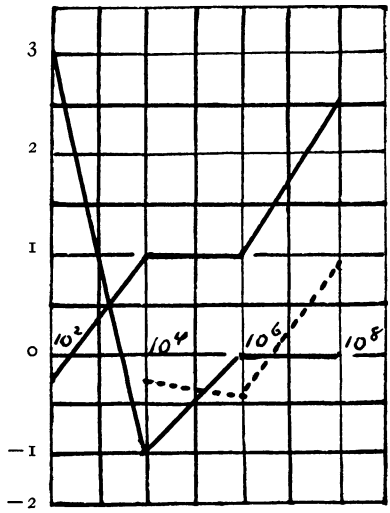


CHART 15.—Epiphanin reaction. Myelogenous leukemia, roentgen ray burn.

——— Simultaneous titrations, antigen I, four-glass method.

- - - - - - - - Antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating 49^{-9} drops, after heating, 50^{-4} drops, control, 49^{-8} drops.

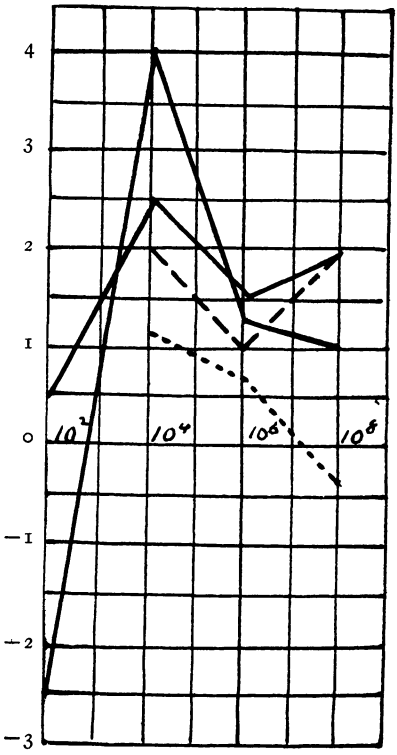


CHART 16.—Epiphanin reaction. Myelogenous leukemia, roentgen ray therapy.

——— Simultaneous titrations, two curves, antigen I, four-glass method.

- - - - - - - - Antigen I plus antikenotoxin, four-glass method.

. . . . . Antigen I, single-glass method.

Meiostagmin reaction of same serum: antigen II, before heating 47^{-7} drops, after heating, 47^{+1} drops, control, 47^{-4} drops.

that of von Angerer and Stoetter. Their titrations were made with a barium hydrate solution much more dilute than that employed in the epiphanin reaction as outlined by Weichardt. The avidity of their reagent for CO_2 (the CO_2 of the air is the one great source of error in the epiphanin reaction) must therefore have been a great many times less intense than that of the concentrated reagent ordinarily employed in the reaction. Unfortunately, however, it will be seen that as the range of error decreased with this modified technic so also did the curves plotted from the serum reactions recede, with very few exceptions remaining within the previously established range of error for the single-glass method. In this respect these results resemble those of Korff-Peterson and Brinkmann.[1] The range of error at no time was as great as that obtained by these workers. This in all probability is due to the fact that they may have worked with the mikra-pipette. In the latter half of my series, both the single- and four-glass methods were employed. Double titrations with the four-glass method were made simultaneously for this portion of the series. In no single instance, however, were two like curves obtained by the same method for the same serum and antigen mixtures. The addition of Weichardt's antikenotoxin[2] to my antigens did not produce reactions any more marked than those obtained with plain antigens. All sera used in this reaction were 48-hour sera. From the study of these titrations, Rosenthal's results like those of this series would also appear to be within the range of error.

CONCLUSIONS.

A decidedly negative meiostagmin reaction is of more value than a positive one and may be considered of some weight in ruling out carcinoma. A moderately or even strongly positive reaction is not necessarily indicative of malignant tumor.

The epiphanin reaction is valueless in the diagnosis of malignant tumors. The range of error determined by the blind titrations in a measure also explains the results obtained by other workers who have employed this reaction in the diagnosis of diseases other than carcinoma.

[1] *Ztschr. f. Hyg. u. Infectionskrankh.*, 1912, 72, p. 343.

[2] *Ztschr. f. Immunitätsf.*, 1912, 13, p. 383.